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Sodium Chloride Effect on Autoxidation of the Lard Component of a Gel

SUMMARY—Stable gels composed of lard, sodium carbomethoxy cellulose and water were used for the examination of factors involved in the pro- and antioxidant activities of sodium chloride, other inorganic salts, heme compounds, meat fractions and other additives. Autoxidation processes were evaluated by peroxide and monocarbonyl determinations. The solid translucent gels, in which additives had been incorporated, were stored frozen, freeze-dried or allowed to oxidize without physical change. The hydrated gels were well aerated in preparation and oxidized in the dark at a convenient pace at 20°C. When the gel was freeze-dried, a sponge-like structure was obtained which, after an induction period, autoxidized rapidly. Freezer-stored gels autoxidized at a rate roughly similar to freezer-stored meat. Sodium chloride had a direct pro-oxidant action on the lard of freezer-stored and dehydrated gels. Hydrated gels containing NaCl when stored at 20°C had an inhibiting autoxidation pattern somewhat similar to the quantitative influence of NaCl on pH. Ethylene-diaminetetraacetate (EDTA) had a powerful antioxidant influence. Sodium chloride accelerated heme catalysis regardless of the presence of antioxidant or chelator. Interesting differences in monocarbonyl patterns and monocarbonyl/peroxide ratios as influenced by additives and moisture content of the gels were observed.

INTRODUCTION

THE MECHANISM of the pro-oxidant effect of NaCl on triglycerides in meat has not been completely elucidated. Background and earlier research efforts on this anomalous meat preservation effect Mabrouk et al., 1960; Watts, 1962; Castell et al., 1965) have been recently reviewed (Ellis et al., 1968a). Ellis et al. (1968) probed factors involved in the pro-oxidant action on pork cuts and mixtures of backfat and lean. It was found that NaCl, either acting directly or indepedently and/or by sensitizing catalysts in the meat, seemed to have the effect of changing some of the autoxidation characteristics of the triglycerides.

The main problems appear at this time to concentrate to the following objectives: (1) Investigation of the existence of an independent oxidative effect of NaCl on fats; (2) investigation of the effect of NaCl on powerful catalysts such as heme pigments; and (3) observation of the characteristics of fat autoxidation promoted by the heme pigments in terms of hydroperoxide breakdown, specificity of fatty acid attack and monocarbonyl compound patterns.

For such a study a rapid means of fat autoxidation was required which would permit the isolation of factors responsible for glyceride fatty acid breakdown in meat. Bishov et al. (1960) used sodium carbomethoxy cellulose to considerable advantage in the preparation of stable emulsions for study of model systems under dehydrated conditions. This model system was utilized in this investigation.

EXPERIMENTAL

Materials

Sodium carbomethoxy cellulose gum (CMC-7H3SF) was donated by Hercules Powder Co.

Hemoglobin (Hb), myoglobin (Mb), cytochrome C, and tetrasodium ethylenediaminetetraacetate (EDTA) were obtained from Nutritional Biochemicals Corp.

Lard was rendered as described by Gaddis et al. (1966). Four lots were used in the experiments discussed in this paper. The number of separate experiments carried out amounted to 64.

Solid translucent gels were made up with combinations of 1:2:40 by weight of CMC, lard and water. The following sequence was used in preparing the gels: Water was placed in a Waring blendor and additives, such as NaCl or Hb, were combined with the water; the lard was added and the mixture was blended briefly. CMC was added and the components were blended thoroughly for 1 min. The resulting gel was used in the hydrated form or freeze-dried. The freeze-dried products were ground in the Waring blendor before storage. Controls were run in each experiment and experiments repeated several times to check reproducibility.

Oxidation of the freeze-dried and hydrated gels was followed by determination of peroxide values (PV) by the method of Kenaston et al. (1955). PV's were calculated as meq/1,000 g lard. Samples of fat for iodometric measurement of PV were obtained by extraction of the gel with chloroform.

Monocarbonyls were determined as 2,4-dinitrophenylhydrazone derivatives. Fat and oxidation products were extracted from the oxidized gels with hexane. An aliquot of the hexane extract, representing 5.0 g of lard, was reacted with 2,4-dinitrophenylhydrazone on the Schwartz column (Schwartz et al., 1963). The resulting carbonyl derivatives were separated from the lard and fractionated to simple monocarbonyl derivatives as described by Schwartz et al. (1963). Milder vacuum distillation methods (Gaddis et al., 1966) could not be used because of the tenacity of the gel which made quantitative removal of the free volatile carbonyls uncertain. However, the method employed was considered suitable for comparative purposes.

Recovery experiments of known monocarbonyl compounds indicated salvage of micro quantities in the range of 85–90%. Monocarbonyl hydrazones were separated into alkanal, alk-2-enal, and alk-2,4-dienal classes by the method of Gaddis et al. (1959). This data enabled determination of proportions of classes of monocarbonyl compounds. Each class was separated into individual compounds and the compounds were estimated as described by Ellis et al. (1959).

RESULTS & DISCUSSION

Hydrated gels

The hydrated gels autoxidized rapidly at 20°C. Storage in the dark was necessary to obtain a moderate and experimentally convenient rate of oxidation. Table 1 indicates the precision obtained with 23 control samples of hydrated gel representing four different lard lots. The unit of time employed was one day intervals. The greatest variability in time was ±1.17 days at PV 40 with an overall average of ±0.77. Precision was very good during the induction period and in the early stages of autoxidation. For samples set up simultaneously, the pre-

Table 1—Precision of gel method: Expressed in variability in Peroxide values; average deviation from mean.

Days of storage	Peroxide values
5	±0.31
10	±0.43
20	±0.85
30	±1.0
40	±1.17
50	±0.82
60	±0.82

have the power of reducing the effect of oxidation inhibiting amounts of Hb or heme pigments.

Banks et al. (1961) reported that ytochrome C in $1.75 \times 10^{-5}M$ concentration was an active catalyst, but at higher concentrations ($3.5 \times 10^{-5}M$) it inhibited autoxidation. The effect was considered due to peroxide breakdown to inactive compounds. Lewis et al. (1963) have observed similar inhibitory effects with Hb, hemin and tissue homogenates.

The mechanism of the effect of NaCl in counteracting inhibitory amounts of Hb is not clear. There is some indication that the key to the pro-oxidant effect of NaCl could be in part due to its pH-lowering action. However, Banks et al. (1961) have observed that cytochrome C was particularly active at pH's above 6.0. Wills (1965) has shown that pH has little influence on heme catalysis, but a marked effect on metal catalysis.

Uncured meat, stored in the freezer, oxidizes very slowly although abundant quantities of heme pigments are present. The addition of NaCl causes a comparatively rapid rate of autoxidation in freezer-stored meat. The effect of NaCl on unfrozen meat has not been determined because of masking deteriorative changes due principally to microoragnisms. The influence of NaCl on meat tissue is probably manifold; however, a direct effect of NaCl on potential pro-oxidant catalysts operates. Lewis et al. (1963) observed that homogenates of liver, heart and spleen were catalytic of linoleic acid oxidation in dilute suspensions and inhibitory in high concentrations.

These results permit the postulation that the pro-oxidant effect of NaCl on meat may be due in some degree to a modification of the inhibitory action of relatively high concentrations of heme pigments and other components.

Freezer storage of hydrated gels

The hydrated gel remained physically stable when frozen and appeared applicable for use as a model system in the study of autoxidation factors under conditions of freezer storage. A comparison was made of the rate of peroxidation in the hydrated gel with and without 2.27% NaCl at 25°F. Autoxidation was extremely slow and reminiscent of freezer-stored meat in its pace. Appreciable PV's did not appear until after several months of storage. At the termination of six months, the NaCl-treated sample had a PV of 45 as compared to 5 for the control.

Thus, freezing converted an oxidation-inhibiting influence of NaCl to a pro-oxidative function, and this would seem to represent a direct action by the NaCl. Such experimental conditions should prove valuable in further study of factors influencing autoxidation in freezer-stored meats.

Storage of freeze-dried gels

Freeze drying the gels produced a spongy solid which could be ground into fluffy, fine particles. This preparation oxidized readily. However, induction periods were frequently longer than those of the hydrated gels.

Sodium chloride accelerated the autoxidation of lard in the freeze-dried solid. A sample containing 25% NaCl (2.3% NaCl as hydrated) reached a PV of 30 in 4 days as compared to a control's time of 9 days. This was a direct effect unaided by any agent unless it were trace metals. However, the possible influence of trace metals appears to be ruled out by the use of EDTA. Use of 2.59% EDTA (0.093% EDTA as hydrated) on the control increased the time to reach PV 30 from 9 to 14 days. Employment of 2.59% EDTA and 24.5% NaCl reversed the time from 14 to 10 days. This demonstrates that the chelator did not eliminate the pro-oxidant effect of NaCl.

Thus, inhibition by NaCl existed in the hydrated form and acceleration under dehydrated conditions (including frozen). Influence of pH would not be a factor here. These results agree with the research findings of Chang et al. (1950).

In other experiments results were essentially similar to those reported for the hydrated gels. Hb and Mb accelerated the autoxidation and were sensitized to a greater acceleration by NaCl. These results seem to definitely indicate that pH is not the only factor involved in the action of NaCl on heme pigments and meat.

An interesting and significant result was obtained when pork lean juice, pork lean aqueous extract and pork lean were incorporated with the lard gel. These experiments were set up only for the freeze-dried gel to avoid

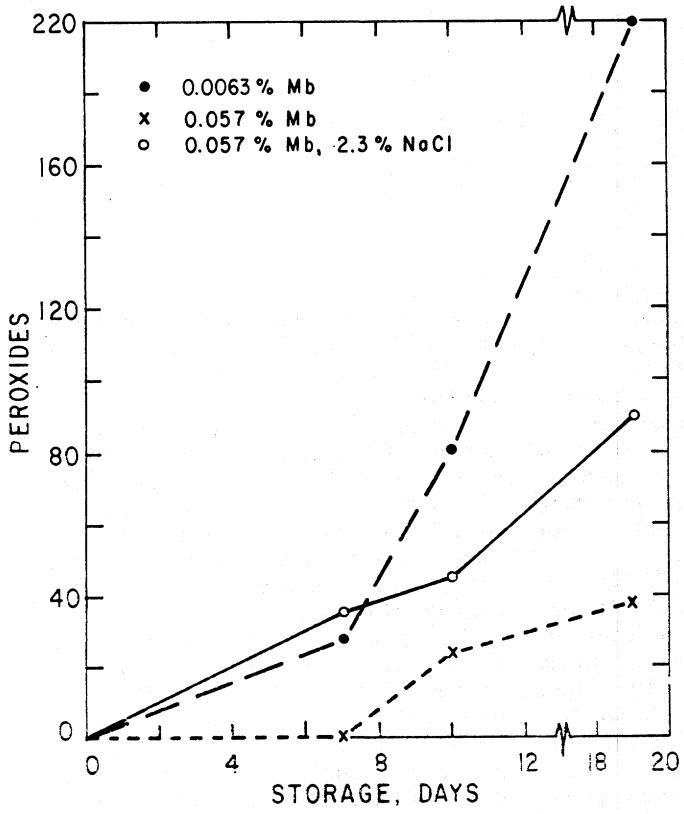

Fig. 3—Effect of NaCl on oxidation inhibiting amounts of Hb in a hydrated gel.

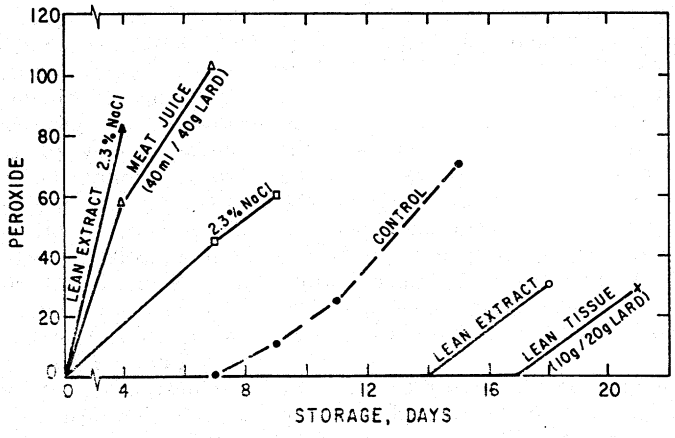

Fig. 4—Effect of meat fractions and NaCl on the autoxidation of freeze-dried gels.

better understanding of the pro-oxidant activity of NaCl.

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